

MICROBIAL PROCESSES

AFFECTING THE BIOREMEDIATION OF METALS AND RADIONUCLIDES

In Sections II through IV we described the basic ingredients for the bioremediation of metals and radionuclides — microbial metabolism, chemical speciation and valence status, and transport processes. Here we describe how scientists and engineers believe these ingredients can be combined to bioremediate contaminated sediments and groundwater. However, because bioremediation of metals and radionuclides relies on a complex interplay of these processes and our understanding of them is just developing, our descriptions are often more qualitative than quantitative. Over the next decade we expect to gain a fundamental, mechanistic understanding of the coupling between microbial metabolism, chemical reaction, and transport — and how these work together to bioremediate metals and radionuclides. However, for now researchers are building on

processes that can be understood and observed with the tools currently available.

Bioremediation of metals and radionuclides is achieved through biotransformation. Like biodegradation of organic components, it involves the breaking and creating of chemical bonds that alter the molecular species of the contaminant. This leads to changes in the solubility, sorption characteristics, transport properties, and toxicity of the metal or radionuclide. There are at least three categories of microbial processes that can influence the toxicity and transport of metals and radionuclides: biosorption and bioaccumulation; biologically catalyzed redox reactions that lead to immobilization; and biologically catalyzed solubilization. Each of these provides the potential for either mobilizing or immobilizing metallic and radioactive contaminants in the environment.

BIOACCUMULATION AND BIOSORPTION

Microorganisms can physically remove heavy metals and radionuclides from solution through either bioaccumulation or biosorption. Bioaccumulation is the retention and concentration of a substance by an organism. In bioaccumulation, metals are transported from the outside of the microbial cell, through the cellular membrane, and into the cell cytoplasm, where the metal is sequestered and therefore immobile (Figure 5.1.a).

Biosorption does not consume cellular energy. Positively charged metal ions are sequestered — primarily through the adsorption of metals to the negative ionic groups on cell surfaces, the polysaccharide coating found on most forms of bacteria, or other extracellular structures such as capsules or slime layers (Figure 5.1.b). Binding sites on

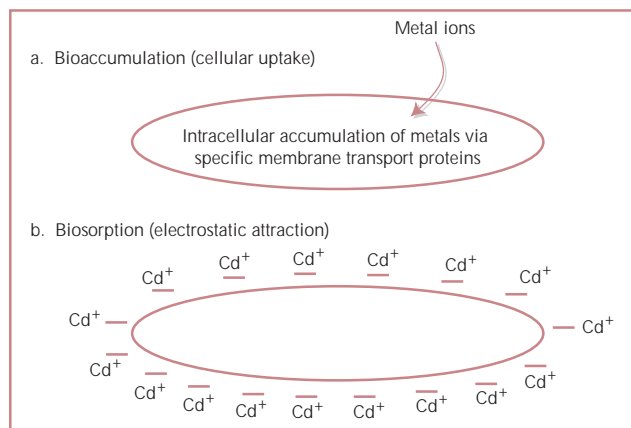


Figure 5.1. Accumulation of heavy metals and radionuclides by bacteria. (a) Metabolically active cells that express metal transport proteins can sequester metal ions intracellularly. (b) Negatively charged bacterial surfaces electrostatically attract metal cations.

microbial cell surfaces usually are carboxyl residues, phosphate residues, S–H groups, or hydroxyl groups. The amount of metal biosorbed to the exterior of bacterial cells often exceeds the amount predicted using information

about the charge density of the cell surface. Scientists have demonstrated that charged functional groups serve as nucleation sites for deposition of various metal-bearing precipitates.

BIOLOGICALLY CATALYZED REDOX REACTIONS THAT LEAD TO IMMOBILIZATION

Metal-reducing microorganisms can reduce a wide variety of multivalent metals that pose environmental problems at many DOE facilities. The heavy metals and radionuclides subject to enzymatic reduction by microbes include but are not limited to uranium (U), chromium (Cr), and technetium (Tc). Direct enzymatic reduction

involves use of the oxidized forms of these contaminants as alternate electron acceptors. The oxidized forms of these three metals are highly soluble in aqueous media and are generally the most mobile species in aerobic groundwater, while the reduced species are highly insoluble and precipitate from solution. Direct enzymatic

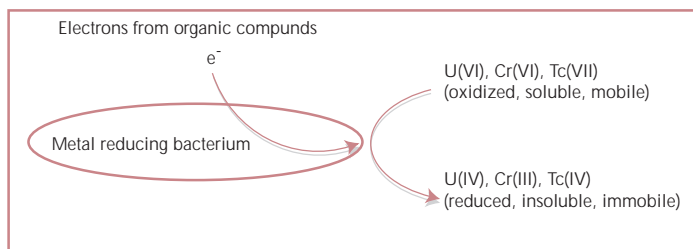


Figure 5.2. Direct enzymatic reduction of soluble heavy metals and radionuclides by metal-reducing bacteria. Nonhazardous organic compounds, such as lactate or acetate, provide electrons used by these microorganisms. Note, however, that if complexed the reduced species may become mobile.

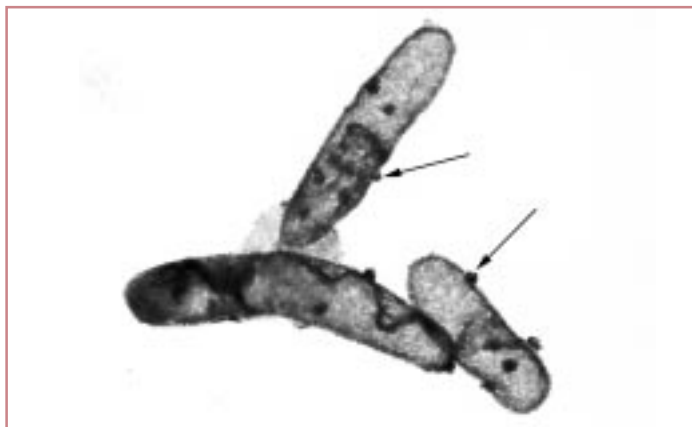


Figure 5.3 This image demonstrates the ability of *Shewanella putrefaciens*, a bacterium isolated from the deep subsurface, to enzymatically reduce and precipitate technetium (dark regions on the cell surface indicated by arrows). This phenomenon offers potential for in situ treatment of radionuclides at DOE sites. Image taken by J. A. McKinley and A. E. Plymale as part of a project led by R. E. Wildung and Y. Gorby at Pacific Northwest National Laboratories.

reduction of soluble U(VI), Cr(VI), and Tc(VII) to insoluble species has been documented and is illustrated in Figure 5.2. Extracellular precipitation of enzymatically reduced Tc by *Shewanella putrefaciens* is illustrated in Figure 5.3. Studies have also found that bioreduction of hexavalent chromium can occur through aerobic and anaerobic conditions. A number of Cr(VI)-reducing microbial strains have recently been isolated from chromate-contaminated waters, soils, and sediments, including *Oscillatoria* sp., *Arthrobacter* sp., *Agrobacter* sp., *Pseudomonas aeruginosa* S128, *Chlamydomonas* sp. (algae), *Chlorella vulgaris* (algae), *Zoogloea ramigera*, and anaerobic sulfate-reducing bacteria.

Metal-reducing organisms reduce uranyl carbonate, which is exceedingly soluble in carbonate-bearing groundwater, to highly insoluble U(IV), which precipitates from solution as the uranium oxide mineral uraninite. Recently, scientists have had success in microbial binding of U(VI), which is then converted by the living cells to U(IV) and precipitated intracellularly. A wide range of bacteria, including *Enterobacter cloacae* and all known metal-reducing bacteria, reduce the highly soluble chromate ion to Cr(III), which under appropriate conditions precipitates as Cr(OH)₃. Metal-reducing bacteria also reduce oxidized technetium, Tc(VII) (which

can be found in soluble sodium pertechnetate) to Tc(IV), forming the Tc oxide mineral TcO_2 .

Although some microorganisms can enzymatically reduce heavy metals and radionuclides directly, indirect reduction of soluble contaminants may be more feasible in natural sedimentary and subsurface environments. This indirect immobilization could be accomplished by metal-reducing and sulfate-reducing bacteria. This can be achieved by coupling the oxidation of organic compounds or hydrogen to the reduction of ferric iron [Fe(III)], Mn(IV), or sulfate (SO_4^{2-}). Iron(III) is reduced to iron(II), manganese(IV) to manganese(II), and SO_4^{2-} to hydrogen sulfide (H_2S). The reduced form then chemically interacts with the contaminants and forms separate or multicomponent insoluble species.

The most reactive of these reduced forms are Fe(II) and H_2S . Ferrous iron [Fe(II)], which is generated by the enzymatic activity of iron-reducing and some fermentative bacteria, can reduce multivalent metals such as uranium, chromium, and technetium (Figure 5.4.a). The reduced forms of these metals are insoluble and can either precipitate as reduced oxide or hydroxide minerals or coprecipitate with Fe(III) minerals that form during the reoxidation of Fe(II). In coprecipitation, elements become incorporated in metal oxide minerals as they precipitate from solution. Although the use of Fe(II) as an electron donor for reduction and precipitation of multivalent metal contaminants has been examined in the laboratory, field tests evaluating its potential as a remediation technology have not been conducted.

Sulfate-reducing bacteria also may be stimulated to produce a chemically reactive redox barrier (Figure 5.4.b). Hydrogen sulfide generated by sulfate-reducing bacteria could chemically reduce the contaminant directly, or indirectly in the case of sulfide minerals such as pyrite that would

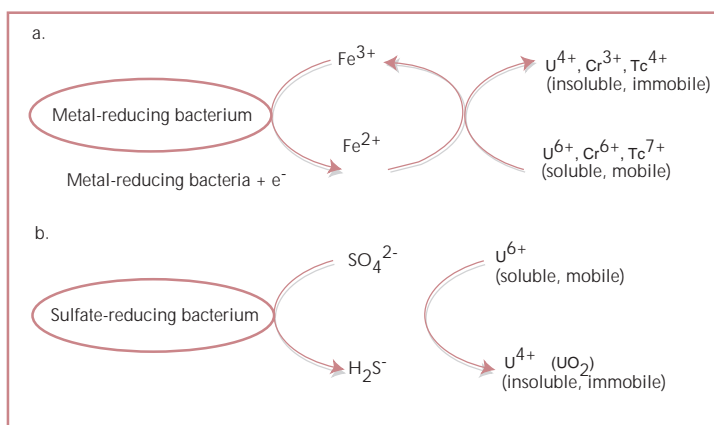


Figure 5.4. Indirect mobilization of heavy metals and radionuclides by (a) metal-reducing and (b) sulfate-reducing bacteria.

be chemically stable for extended periods of time.

Synthetic chelators such as EDTA and NTA can form stable, soluble complexes with heavy metals and were commonly used as cleaning agents during industrial processing of nuclear fuels throughout the DOE complex. Metal-chelate complexes have entered the environment and may migrate freely in groundwater. When conditions necessitate immobilization of the contaminant, one approach for limiting the migration of the metal is to biodegrade the organic ligand (Figure 5.5). The resulting free metal ions are likely to adsorb to mineral surfaces or form oxide mineral precipitates that would transport poorly in groundwater. A number of EDTA- and NTA-degrading organisms have been identified. However, little is known about the enzymes that catalyze the degradation reactions and how these reactions proceed in the environment. In one study, microbial degradation of EDTA by the environmental isolate BNC1 was influenced by the complexed metal. Cobalt(II)-EDTA, cobalt(III)-EDTA, and nickel(II)-EDTA complexes were not degraded, whereas copper(II)-EDTA and zinc(II)-EDTA complexes were. Similar fundamental research focusing on the mechanisms of enzymatic degradation of synthetic chelators is expected to provide useful information for including these enzymes in engineered bioremediation technologies.

BIOLOGICALLY CATALYZED REDOX REACTIONS THAT LEAD TO SOLUBILIZATION

Solubilization of biosorbed and coprecipitated metals also can occur by direct or indirect microbial processes. However, the solubilization of toxic heavy metals and radionuclides from coprecipitates requires at least partial solubilization of the oxide mineral itself. Bacteria can catalyze the dissolution of iron oxide minerals by direct and indirect mechanisms. As previously described, metal-reducing bacteria enzymatically reduce and, under proper environmental conditions, solubilize oxide minerals. Such dissolution reactions have been shown to release cadmium, nickel, and zinc into solution during reduction of goethite (a form of iron oxide) by an anaerobic *Clostridium* species. Direct reduction of iron oxide precipitates by metal-reducing bacteria has been shown to release soluble radium from uranium mine tailings. Direct enzymatic reduction of iron oxides provides potential for releasing a wide range of heavy metals and radionuclides that were coprecipitated and immobilized in subsurface sediments. However, not all metals have been tested and more research is needed in this area.

Metal-reducing bacteria also can promote the mobilization of insoluble forms of some heavy metals. For example, PuO_2 exists as a solid in contaminated environments. It has been demonstrated that metal-reducing bacteria solubilized PuO_2 in the presence of the synthetic chelator NTA. It is thought that the bacteria reduced insoluble Pu(IV) to Pu(III) , which was then complexed by NTA. This process may provide a means of mobilizing Pu from contaminated soils

and sediments. This could be a step in the removal of this highly toxic radionuclide from the environment. However, this approach has not been tested in the field.

Organic acids formed by the metabolic activity of microorganisms can lower the pH of the system to values that interfere with the electrostatic forces that hold heavy metals and radionuclides on the surface of iron or manganese oxide minerals. Displacement of cations by hydrogen ions may lead to the solubilization of the surface-associated metal. In some cases the organic metabolites also serve as chelating agents that can form soluble metal–ligand complexes. These chelating agents, such as dicarboxylic acids, phenolic compounds, ketogluconic acids, and salicylic acids, have been shown to promote the dissolution of a wide range of heavy metals and radionuclides, including PuO_2 , and copper, uranium, thorium, and nickel oxides, and can accelerate the movement of metals in soils and sediments.

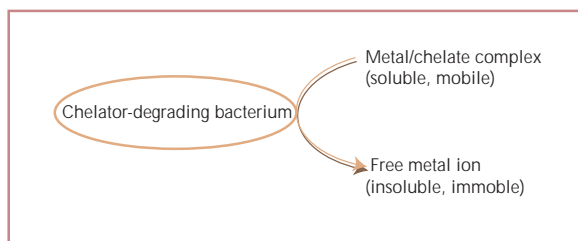


Figure 5.5. Immobilization of radionuclides and heavy metals by enzymatic degradation of organic chelators, such as EDTA and NTA.